

function MLH1 protein, while the pC9MLH1 stop produces the non-functional truncated MLH1 polypeptide. The polyPNP and PNP vectors are described in Fig. 4. The polyPNP contains a 21 base out-of-frame polyA tract inserted after codon 2 of the bacterial PNP gene which results in a truncated polypeptide (Sorscher, EJ, et.al. Gene Therapy 1:233-238, 1994). The polyPNP contains a 20base in-frame polyA tract inserted after condon 2 of the bacterial PNP gene which results in a full-length functionally active PNP protein. Both the polyPNP and PNP gene have a hemagglutinin (HA) epitope fused in-frame at the C-terminus followed by a termination codon. The polyPNP and the PNP gene was constructed by polymerase cahin reaction using a sense primer : 5'-
ccaagcttagaccaccatggcaaaaaaaaaaaaaaaaaaaatcgctacccacacattaatgc-3' (SEQ ID NO: 1), where the polyA tract is underlined while the primer for PNP contains 1 less A in the polyA tract. The antisense primer for both constructs is
5'ataagaatgcggccgctatccttagctagcgtaatctggaacatcgtaagcgtaatctggaacatcgctactctttatcgcccagcag-3' (SEQ ID NO: 2). DH5α bacterial DNA was used a template for amplification. The modified PNP gene was produced by amplification USING 95°C FOR 30 SEC, 54°C FOR 1 MINUTE, 72°C for 1 min for 25 cycles in buffers as previously described (19). Amplified genomic inserts were cloned into T-tailed vectors (TA cloning, Invitrogen) and recombinant clones were sequenced to identify vectors with correct nucleotide sequences. PNP fragments were then subcloned into the KpnI-XhoI sites of the pCeP4 vector (Invitrogen) using sites from the TA cloning vector polylinker. Recombinant PNP expression vectors were sequenced to ensure sequence authenticity using internal primer sequences.

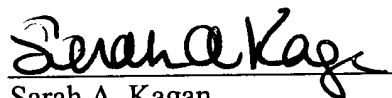
On page 9, the second full paragraph please substitute the following paragraph:

Figure 7. Diagram of the genetically altered purine phosphorlyase (PNP) gene with an out-of-frame poly A tract inserted in the N-terminus (referred to as polyPNP) (SEQ ID NO: 3). This gene

encodes for a non-functional PNP gene. When the polyA tract is randomly altered by a defective MMR, the tract is shifted and allows for the production of a functional PNP gene. PNP can convert the non-toxic prodrug 9-(β -D-2-deoxyerythropentofuranosyl)-6-methyl-purine (referred to as MPD) to the toxic 6-methyl purine analog (referred to as (MP). The construct has a hemagglutinin (HA) tag at the C-terminus for western blot analysis. A control construct with an in-frame polyA tract encoding a full-length polypeptide (SEQ ID NO: 4) is shown on the bottom.

Respectfully submitted,

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